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## **LDRD Final Report on Nanovehicle Light-driven Propulsion**

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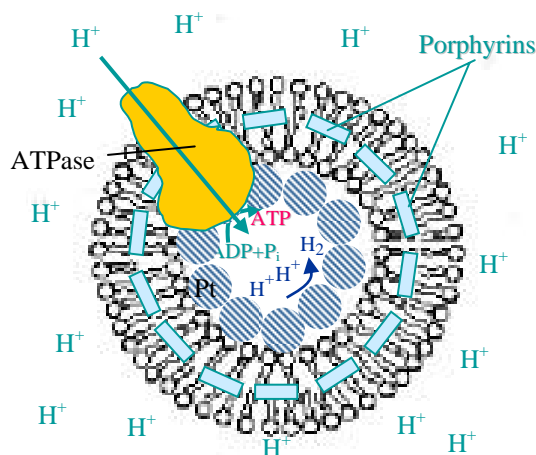


## **Abstract**

Having demonstrated the possibility of constructing nanoscale metallic vehicular bodies as described in last year's proposal, our goals have been to make uniform preparations of the metallized lipid assemblies and to determine the feasibility of powering these nanostructures with biological motors that are activated and driven by visible light. We desired that the propulsion system be constructed entirely by self-assembly and powered by a photocatalytic process partially already built into the nanovehicle. The nanovehicle we desire to build is composed of both natural biological components (ATPase, kinesin-microtubules) and biomimetic components (platinized liposomes, photosynthetic membrane) as functional units. The vehicle's body was originally envisioned to be composed of a surfactant liposomal bilayer coated with platinum nanoparticles, but instead of the expected nanoparticles we were able to grow dendritic 2-nm thick platinum sheets on the liposomes. Now, we have shown that it is possible to completely enclose the liposomes with sheeting to form porous platinum spheres, which show good structural stability as evidenced by their ability to survive the stresses of electron-microscopy sample preparation. Our goals were to control the synthesis of the platinized liposomes well enough to make uniform preparations of the coated individual liposomes and to develop the propulsion system for these nanovehicles—a hydrogen-evolving artificial photosynthetic system in the liposomal bilayer that generates the pH gradient across the membrane that is necessary to drive the synthesis of ATP by ATP-synthase incorporated in the membrane. ATP produced would fuel the molecular motor (kinesin) attached to the vehicle, needing only light, storable ADP, phosphate, and an electron donor to be produced by ATP-synthase in the membrane. These research goals appear to be attainable, but growing the uniform preparations of the liposomes coated with dendritic platinum sheeting, a necessary accomplishment that would simplify the task of incorporating and verifying the photosynthetic function of the nanovehicle membrane, has proved to be difficult. The detailed understanding of the relative locations of surfactant and Pt in the liposomal bodies has also forced a change in the nanovehicle design strategies. Nevertheless, we have found no insurmountable obstacles to making these nanovehicles given a larger and longer term research effort. These nanovehicles could potentially respond to chemical gradients, light intensity, and field gradients, in the same manner that magnetic bacteria navigate. The cargo might include decision-making and guidance components, drugs and other biological and chemical agents, explosives, catalytic reactors, and structural materials.

## Introduction

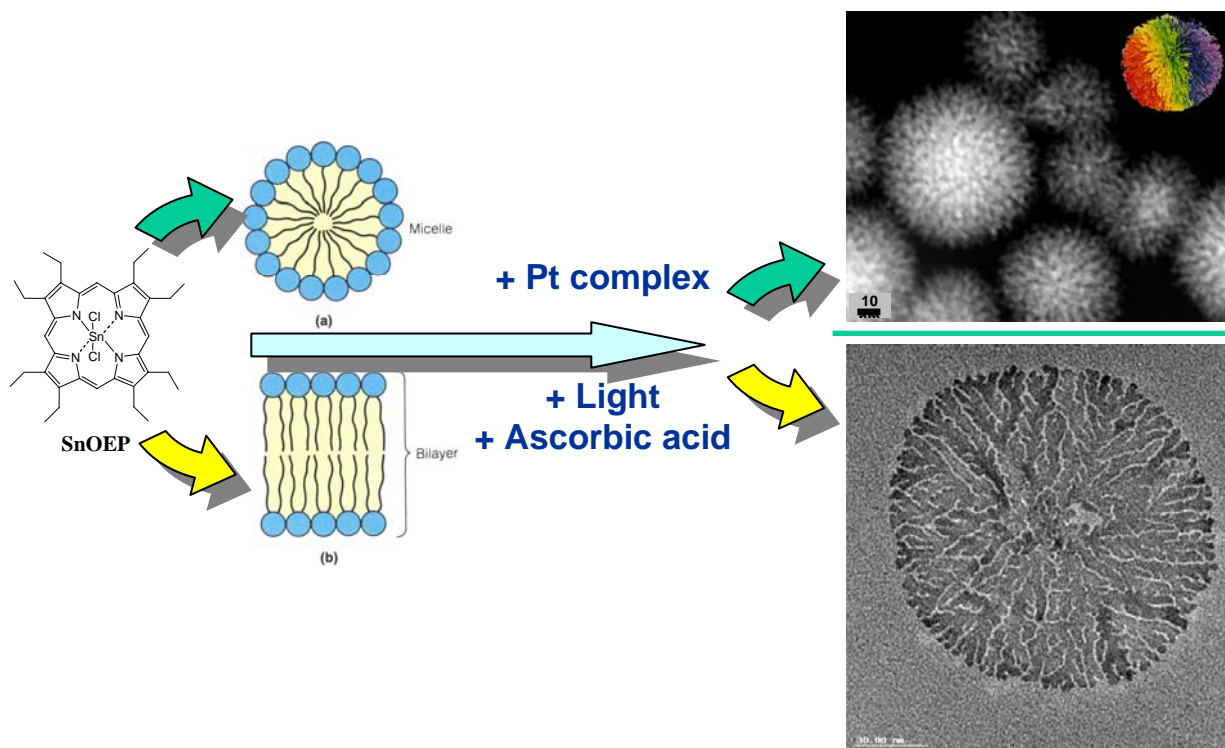
Our original idea of how a nanovehicle and its basic power system would be fabricated entirely by self-assembly and self-compartmentalization processes is illustrated in Fig 1. These nanoscale metallic vehicles would be powered by biological motors attached to the outer surface and activated and driven by visible light. We anticipated that the vehicle's body would be composed of the surfactant bilayer of a liposome, which had been coated with metallic nanoparticles. This metallic shell might be synthesized on the inside (as shown) or the outside surface of the surfactant bilayer of a liposome by a photocatalytic approach being developed at Sandia.



**Fig 1.** The nanovehicle and its power source. Light absorbed by the porphyrin induces  $H_2$  evolution at the Pt particles and a pH increase within the metallized liposome. The pH gradient across the liposomal bilayer is used by ATP synthase, a molecular motor itself, to produce ATP. The ATP produced can power other molecular motors such as myosin and kinesin.

The power components of this nanovehicle, when exposed to light, generate a proton gradient across the bilayer membrane which could be used to drive a motor (e.g., a flagellum or kinesin) to move the vehicle. Incorporating a flagellum or other molecular propulsion systems into liposomes is a challenging problem. The flagellum would have to be properly anchored into the membrane while allowing the flagellum to rotate, and it is not yet possible to reconstitute functioning flagella in this way, though a several groups are working on this problem. Instead, linear motion could be achieved using kinesin motors to carry the metallized liposome by walking on microtubules, a technology being developed at Sandia and elsewhere. Thus, the nanoscale vehicles envisioned are composed of both natural biological components (ATPase, molecular motors) and biomimetic components (vehicle housing, artificial photosynthetic membrane) as functional units. Only light and storable ADP,  $P_i$ , water, and a weak electron donor such as a tertiary amine are required to fuel both motor and delivery-package components. These nanovehicles are constructed entirely by self-assembly and photocatalytic processes.

The ultimate goal is to develop a nanoscale vehicle that can move about to deliver a payload to a specific site. The payload might be (1) the catalytic capability to produce a specific chemical or biochemical, (2) the ability to sense chemical or biological agents, (3) the ability to spatially sort and organize molecules, (4) explosives, (5) nanoscale chemical reactors, and (6) construction materials. If active payloads were constructed properly, different light-driven



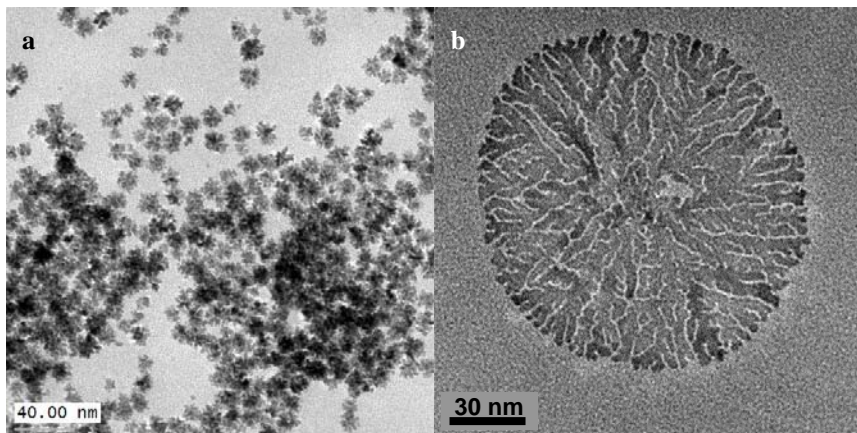
**Fig 2.** Synthesis of platinized micelles and liposomes. In the absence of the Sn porphyrin, growth of Pt dendrites on the micelles is uncontrolled leading to a large size distribution of the spherical dendrites as shown in the upper TEM image. When Sn octaethylporphyrin is added to a solution of sodium dodecylsulfate (SDS) above the critical micellar concentration (green arrows) a uniform distribution of smaller spherical dendrites is obtained when irradiated in the presence of Pt complex and ascorbic acid. TEM images of these small Pt dendrites show that each nanodendrite is a single crystal. Taking the bottom route (yellow arrows) leads to metallized liposomes. The lower TEM image shows a circular dendritic sheet grown on a large liposome; the 2-nm thick sheet only partially covers the liposome in this case.

components within the vehicle might be turned on and off by using different colors of light, giving one the ability to move the nanovehicles and switch on their payloads independently.

Previously, we have demonstrated the feasibility of constructing nanostructures similar to that shown in Fig. 1. The metal employed was crystalline platinum and the surfactant assemblies were sodium dodecylsulfate (SDS) micelles instead of liposomes. Micelles are small surfactant assemblies that are essentially composed of only a single layer of surfactant, as illustrated in Fig 2a. We have shown that it is possible to photocatalytically control the growth of nanodendrites of Pt on the surface of micelles and liposomes; Fig 2 illustrates this synthetic process.

We have now extended this synthesis to the lipid bilayer surfaces of liposomes (Fig. 2b) to create our nanovehicles. Unlike micelles, liposomes are spherical bilayer structures that have an interior and an exterior surface and they enclose solvent in the interior space. Liposomes of between 100 nm and a few microns are easily produced by extrusion of a surfactant solution through pores of a particular size. The synthesis is simple; first a water-insoluble porphyrin is dissolved in the surfactant solution, which is then diluted and sonicated (and extruded) to form an aqueous solution of liposomes containing photocatalyst molecules. Next, a Pt(II) salt

solution, containing mostly  $\text{PtCl}_2(\text{H}_2\text{O})_2$ , and an electron donor (ascorbic acid) are added and the solution containing the desired surfactant assembly is illuminated with light from a tungsten lamp. In this system, photoactivated electron transfer from

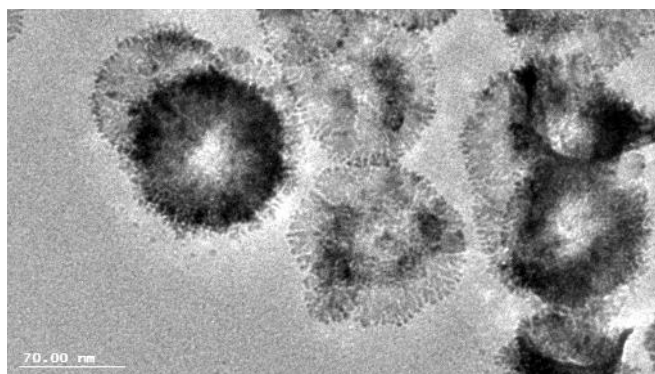


**Fig 3.** TEM images of (a) Pt nanodendrites from micelles and (b) Pt nanosheets grown on liposomes.

ascorbic acid to the porphyrin to the Pt(II) complex results in zero-valent metal being deposited onto the surfactant assembly. The process produces seed nanoparticles which then grow by autocatalytic oxidation of ascorbic acid and reduction of Pt complex. Within 15 minutes, the all metal ions are reduced giving a platinized surfactant assembly containing the still active photocatalyst inside, producing platinum dendrites on micelles and liposomes like those shown in Fig. 2.

The electron microscopy images of these amazing nanostructures (Fig. 3) show that each individual nanostructure is a single crystal, making them extremely sturdy, *e.g.*, they can be dried and re-suspended in solution without disrupting the Pt structure. We have refined our photocatalytic methods for controlling the structure of the platinized liposomes and liposomal aggregates to produce myriad platinum sheet-like nanomaterials, including individual liposomes coated with Pt and Pd dendritic sheeting. The liposomes coated with dendritic Pt nanosheets show potential for serving as the body of our nanovehicles. The best nanostructures for the nanovehicles are formed when there are a few growth centers or seeds, preferably only one seed per liposome with enough of the platinum complex available for this seed to grow sheeting over the entire surface of a liposome.

Spontaneous formation of seeds by slow un-catalyzed reduction of Pt(II) by ascorbic acid in the dark gives only a few seeds, but these are formed continuously over a long time period. Each seed then has a different time to grow before the Pt complex is exhausted giving different degrees of coverage of the liposomes. In the dark or in



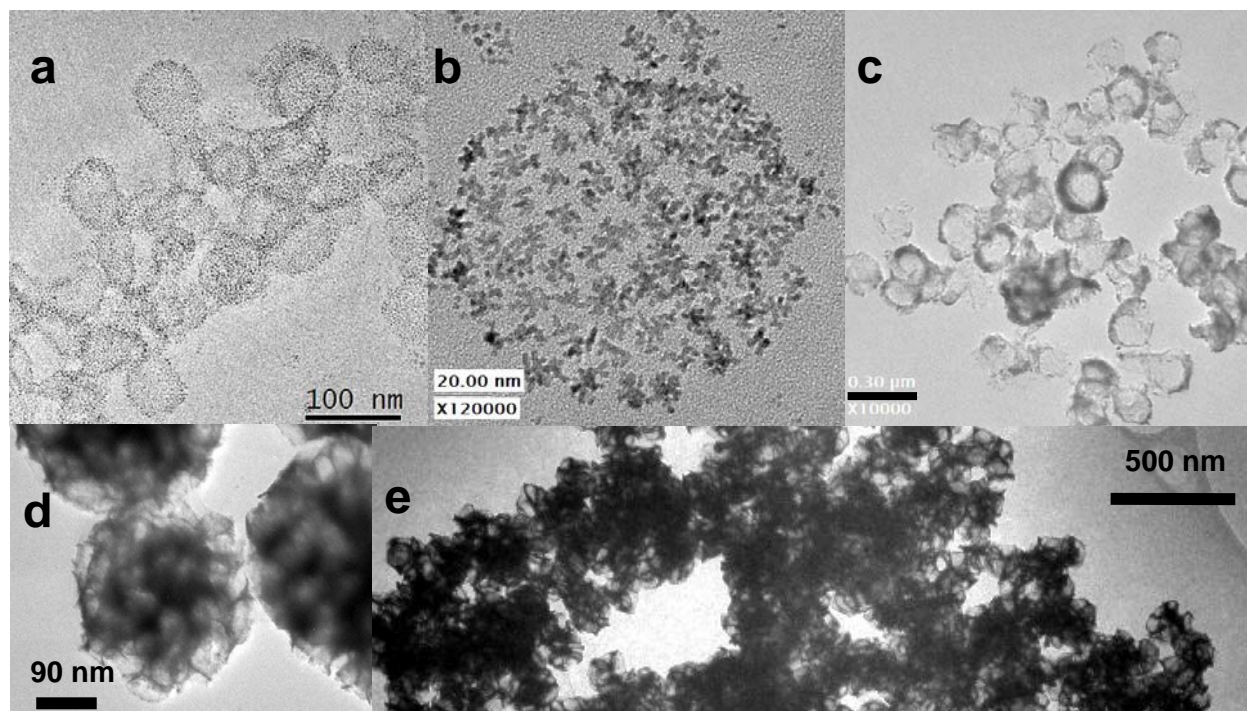
**Fig 4.** TEM images of platinized liposomes. In addition to nanocaps (lighter structures), several nearly fully coated liposomes are evident (darker doughnut-shaped structures).



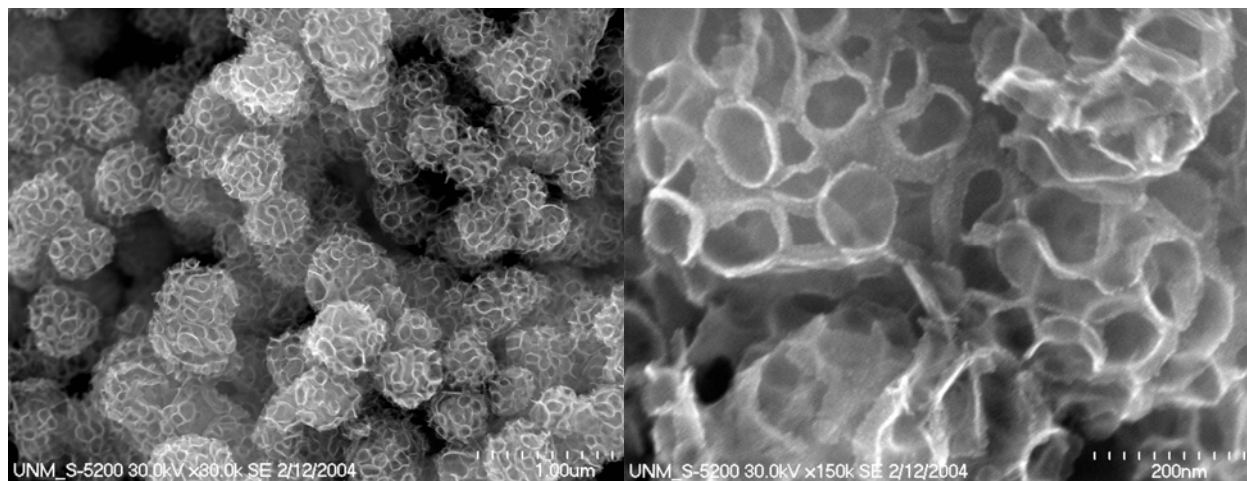
the absence of porphyrin photocatalyst in the liposomal bilayer, the entire surface of the liposome may be covered; both fully and partially coated nanostructures are thought to be observed as flattened structures in TEM images like those in Fig. 4, with the nearly fully coated nanostructure appearing as thick (dark) doughnut-like structures. For spontaneous seeding, the large differences in growth times and thus sizes of the dendritic sheets are expected. It was anticipated that judicious choices of photocatalyst and Pt complex concentration and light exposure would give conditions under which uniformly coated liposomes could be obtained to serve as our nanovehicle bodies.

### Accomplishments.

**Task 1.** *Optimization of the synthesis and structural characterization of the platinized photosynthetic liposomes.* During this year, we sought to more fully characterize the Pt-coated liposomes, especially with regard to controlling the Pt growth, and to determine where the Pt sheeting is located relative to the surfactant layers. The latter is crucial to the development of the desired photochemical propulsion mechanism. We first obtained TEM and SEM images of the platinized liposomes produced under different light exposures and for different porphyrin and Pt concentrations. As the series of TEM images in Fig. 5 show, we can produce liposomes coated with Pt in several different ways including (a) 3-nm particles scattered over the liposomal surfaces, (b) small unconnected 2-nm thick sheet-like dendrites decorating the liposome, (c) Pd dendritic sheets coating entire liposomes, (d) Pt sheets coating aggregated liposomes to form foam-like balls, and (e) dendritic Pt sheets grown together to form foam-like monoliths.



**Fig. 5.** TEM images of various metallized liposomal nanostructures: (a) Pt nanoparticles decorating individual liposomes, (b) small flat Pt dendrites decorating a liposome, (c) Pd dendritic sheets coating entire liposomes, (d) Pt sheets coating aggregated liposomes to form foam-like balls, and (e) dendritic Pt sheets grown together to form foam-like monoliths.

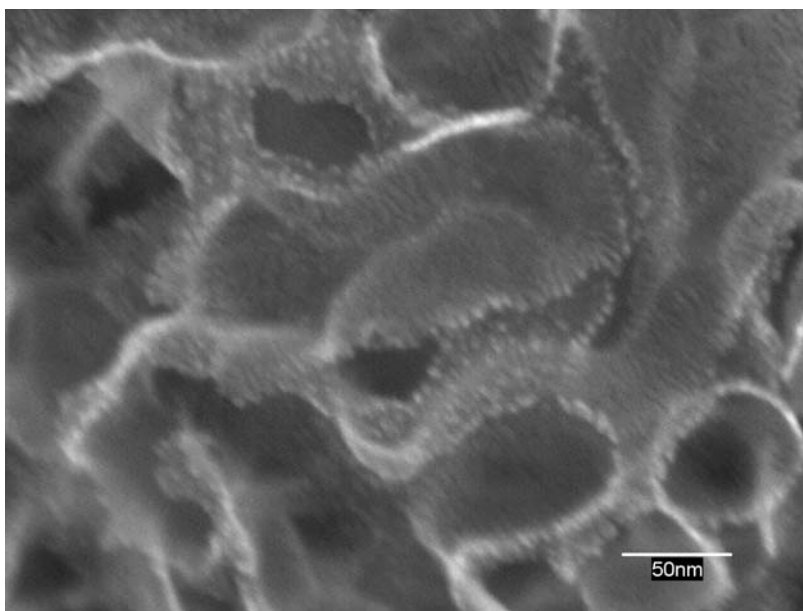


**Fig. 6.** SEM images of various platinized liposomal aggregate nanostructures: (left) Pt foam-like balls formed by dendritic Pt sheets growing over aggregated liposomes and (right) Pt sheet dendrites covering large areas of aggregated liposomes to form foam-like Pt monoliths. Shapes resembling the original liposomes are clearly visible in the image on the right and similar high-magnification images of the foam balls (not shown).

connected dendritic sheets coating the entire liposome, (d) Pt sheeting that follows the surfaces of aggregated liposomes to form Pt foam balls, or (e) large monoliths of foam-like aggregates of coated liposomes. Obviously, the individual platinized liposomes similar to the palladium-coated liposomes shown in Fig. 5c are closest to the objects desired for making the nanovehicles.

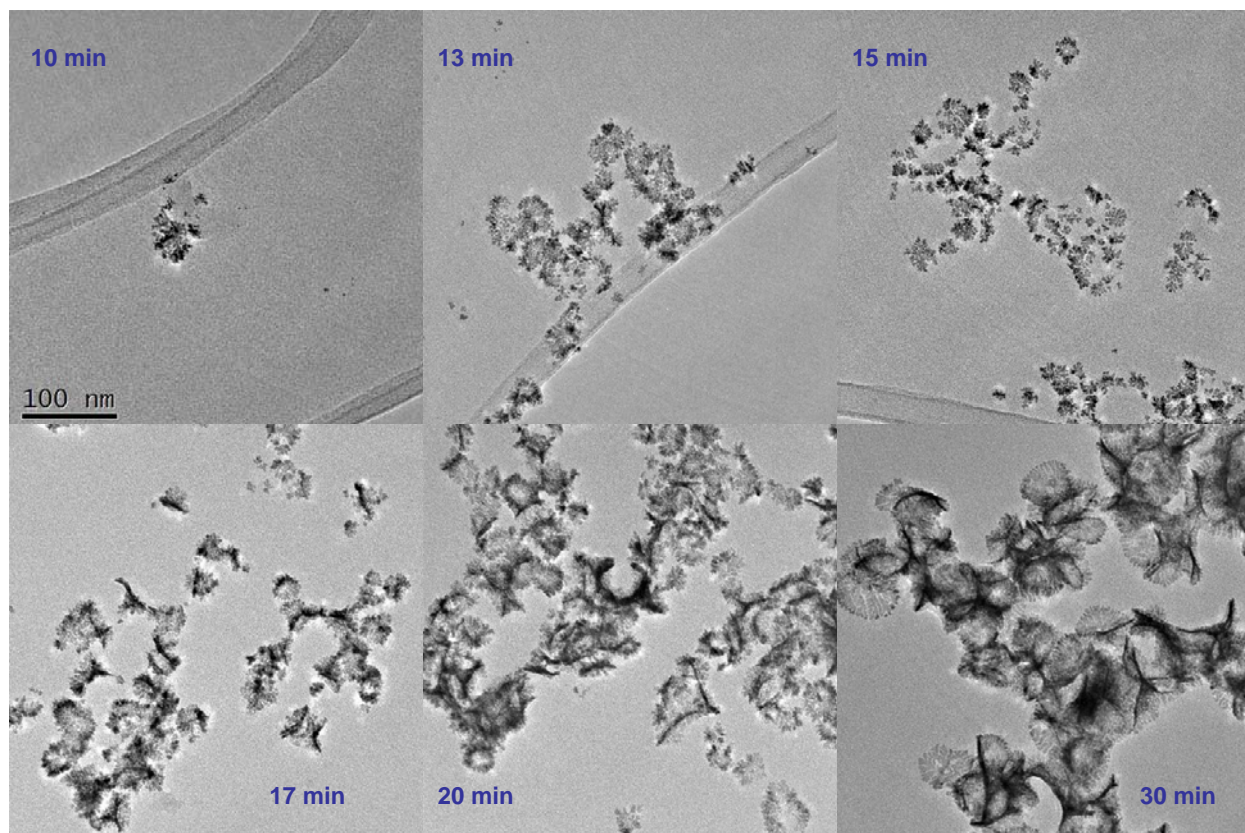
These nanomaterials were further characterized by obtaining high-resolutions SEM images like those shown in Fig. 6. These images clearly show that even in the liposomal aggregates the dendritic Pt sheeting closely follows the shapes of the liposomes without much distortion of the spherical shapes of the liposomes. Fig. 7 shows a high magnification SEM image verifying that the Pt sheeting still retains its dendritic sheet-like nature even in the platinized liposomal aggregates.

The time development of these platinized liposomal structures is shown in Fig. 8. Clearly, to produce pure suspensions of individual coated liposomes using this approach, we must interrupt the development near the 17-minute mark. In addition, we must also gain an understanding of the aggregation mechanism and prevent aggregation in order to



**Fig. 7.** Platinized liposome aggregates, showing the dendritic nature of the Pt sheeting that follows the surfaces of the liposome.

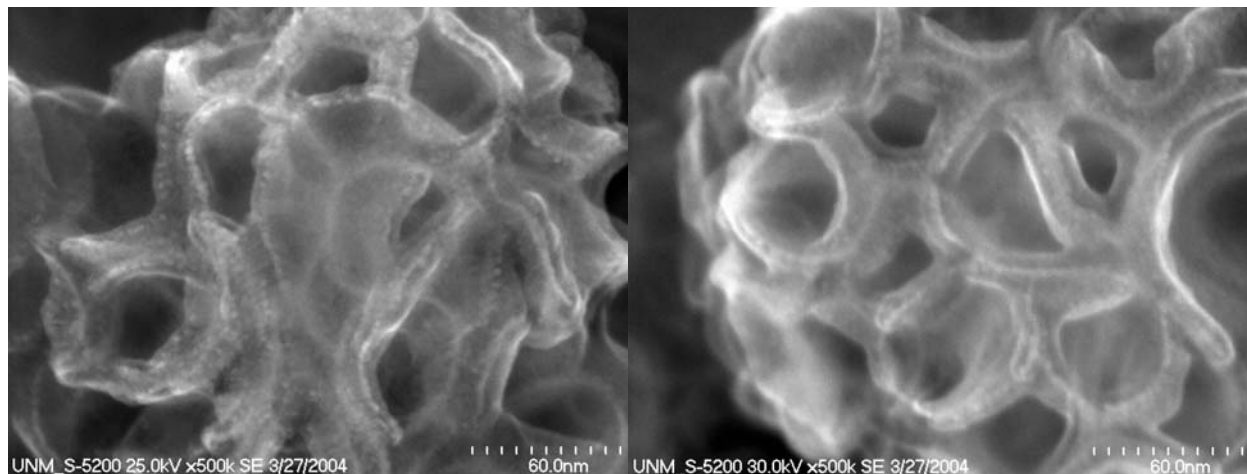




**Fig. 8.** Time development of the growth of the platinized liposome aggregates.

obtain individually coated liposomes. The results shown in Fig. 5c suggest that production of separated platinized liposomes is likely possible when these aggregation and growth processes are fully understood and optimized.

We originally thought that the platinum dendrite grows on the outer surface of the liposomal bilayer. This was a reasonable supposition, but TEM and SEM images of the washed samples of the platinum nanostructure give no indication of the relative dispositions of the surfactant bilayer and the platinum sheet. For such information, we obtained SEM images of unwashed platinized liposomal nanostructures. Unlike the TEM images that show only the Pt structure, SEM images allow the surfactant to be observed because of better contrast with the carbon of the lipid. SEM images of unwashed platinized liposomal nanostructures are shown in Fig. 9. The images show that surfactant coats both sides of the dendritic Pt sheets, suggesting that the Pt dendrite grows within the bilayer of the liposome instead of on the outer surface of the liposome. This view is supported somewhat by the 2-3-nm thickness of the surfactant layer, which corresponds more closely to a monolayer than a bilayer of lipid (which would be ~6 nm thick). It is still possible however that an additional surfactant layer is picked up during the SEM sample preparation and drying process, so this evidence is not conclusive for the platinized liposomes in aqueous suspension. If the platinum sheet is indeed located in the hydrophobic phase of the membrane it



**Fig. 9.** SEM images showing the surfactant on the platinized liposome aggregates. There is a surfactant layer on both sides of the Pt dendritic sheet, suggestion that the Pt dendrite may grow within the bilayer of the liposomes in contradistinction to the illustration shown in Fig. 1.

may be difficult for protons to migrate to the Pt surface to be reduced to molecular hydrogen. On the other hand, if  $H_2$  generation does still occur it will raise the pH inside the liposome more than in the much higher volume of the external environment. This provides a pH gradient which could still be used to make ATP on the inside of the nanovehicle. Unfortunately, the ATP fuel for the kinesin motor may be needed on the exterior of the nanovehicle.

We might ultimately gain additional control by cooling the reaction solution temperature to  $5^\circ$  where spontaneous seeding is shut down. Then, using a brief light exposure to grow seeds and simultaneously initiate growth for all the liposomes, we could obtain the desired uniform growth and coverage of the liposomes. With the spontaneous seed formation shut down, all seeds are photocatalytically formed at the beginning of the reaction and all seeds grow for the same time period giving equal sized platinum structures. This method could give control over the initial number of seed particles and possibly provide one seed per liposome. This might give the desired degree of synthetic control, but for our fuel-generating photosystem, we need many porphyrins in the membrane of each liposome to harvest the light energy. This problem could be solved by addition of more porphyrin to the liposomes after the Pt is deposited.

Given the problems that arose in the making of the nanovehicle bodies and in the detailed structure of the liposome-platinum interface, we focused on solving these issues rather than the tasks associated with attaching the kinesin molecular motor to the surface of the platinized liposome and incorporating the molecular machinery required to fuel the motor. The molecular machinery is already partially present in the form of the photocatalytic porphyrin, which is probably inside the lipid bilayer of the platinized liposomes. This active photocatalytic tin porphyrin will drive the reduction of water to  $H_2$  at the catalytic surface of the platinum dendrites in a well known artificial photosynthetic photoreaction. The  $H_2$  evolution reaction takes up

protons from water, raising the pH inside the nanovehicle (as illustrated in Fig. 1) and thus generating a pH gradient across the liposomal membrane. Whether the pH is raised on the inside or the outside of the liposome depends on whether the platinum is on the inside or outside surface. With the Pt nanosheet between the surfactant monolayers, it becomes unclear whether  $H_2$  can be generated photocatalytically, and certainly the efficiency becomes worse because protons may be taken up from both the interior and exterior regions. The molecular machinery would be completed by incorporating the protein ATP synthase (ATPase) into the membrane. After a light-driven pH gradient is generated, it would produce ATP from ADP and inorganic phosphate ( $P_i$ ) as illustrated in Fig. 1. ATPase has been reconstituted into liposomes by others, although the presence of the platinum sheeting could potentially pose problems. The ATP generated by ATPase using the proton gradient is the renewable fuel of our molecular motor.

The molecular motor we intended to use was a vesicle-attached kinesin molecule, which would propel the vesicle along a microtubule using ATP as the fuel. The kinesin-microtubule system is how vesicles and organelles are transported within cells. The microtubules form a railway system and kinesin connected to vesicles walks along the microtubule rails carrying the vesicle. A single kinesin motor could move a nanovehicle along a microtubule. The problem to be solved next would be how to attach the kinesin motor to our platinized liposome. This might be solved by simple self-assembly, or might require genetic manipulation or chemical modification of kinesin. One method of attachment might be to include a gold nanoparticle on the outer surface and modify the motor protein with a self-assembling thiol group. During this year, the technology for photocatalytically growing such a gold nanoparticle has been developed using Au(I) thiosulfate or thiourea complexes. The enhanced structural integrity of our metallized liposomes is expected to aid in the attachment of the molecular motor.

Finally, we need to make sure that the ATP produced photochemically actually gets to the kinesin in sufficient quantities to run the motor. Being a negatively charged species, ATP cannot ordinarily cross the membrane. This might require the reverse configuration to that shown in Fig 1, *i.e.*, the Pt sheeting should be on the exterior and the ATPase should be inverted in the membrane, producing ATP outside the liposome and near the kinesin motor. The concentration should optimally be close to that in a living cell (2-5 mM). This and the necessity of raising the pH outside of the nanovehicle may require that the external volume be small. This would limit the functionality and conditions under which the nanovehicle could operate. Alternatively, we might retain the organization of the membrane components shown in Fig. 1 and rely on ATP/ADP membrane permeability, which might be altered by inclusion of an anion channel such as mitochondrial porin to provide extra-vehicular ATP for the motor.

Liposomes with incorporated photosystems that pump electrons vectorially across the bilayer membrane are being developed by others using carotenoid-porphyrin-quinone triads, and liposomes with incorporated chloroplast ATPase enzymes have produced both a proton motive force and an inter-membrane potential. Our new approach uses a metallic shell partly to increase stability but, more importantly, as a part of a simpler and efficient photosynthetic ATP-generating chemical system. The previous work on soft liposomes demonstrates the validity of our approach, but lacks the potential advantages offered by our metallized liposomes.

An attractive second generation 'direct-drive' motility system would be a bacterial flagellum and its motor, which is reconstituted directly into the liposome membrane. The flagellum motor does not require ATP, but instead is powered directly by the proton gradient across the plasma membrane. It rotates at about 100 revolutions per second, powered by passive transport of protons back across the membrane. Unfortunately, we do not yet have the ability to reconstitute flagella into liposomal membranes, although this capability may become available in the next few years. In fact, the structural rigidity of the Pt shell of the liposome may aid in the incorporation of natural flagella.

Although the proposed nanovehicle propulsion system is a radically new biomimetic technology and has not been fully evaluated, we believe that our attempt to use the metallized liposomes potentially provides a pathway to light-powered nanovehicles. Being based on an entirely new and robust nanostructure and a new light-driven chemical approach, significant opportunities are presented for producing usable nanoscale vehicles. One of the major risks has now been largely removed with the successful metallization of liposomes to produce apparently robust nanovehicle bodies for our proposed nanobots.

The applications of such nanoscale vehicles seem limitless if these nanobots can be made to translocate and deliver a payload to a specific site. The delivered package might include decision-making and guidance components, drugs and other biological/chemical agents, explosives, and structural materials. The payloads could be biomedical in nature such as delivering catalytic or enzymatic activity capable of correcting biochemical deficiencies. Both biomedical and non-biomedical applications of the ability to transport various sensor capabilities at the nanoscale can be envisioned. The ability to spatially sort and organize molecules at the nanoscale could have interesting environmental and remote construction applications. Imagine mining nanovehicles that hunt and retrieve valuable resources from mineral slurries. They might transport, concentrate, assemble, and ignite explosives payloads on a nanoscale. They might also provide mobile nanoscale chemical reactors for combinatorial procedures or provide chemical power in spatially confined regions. If active payloads were constructed properly, different

light-driven components within the vehicle might be turned on and off by using different colors of light, giving one the ability to move the nanovehicles and switch on their payloads independently. For example, we have been developing chemical- and light-actuated molecular machines based on nickel porphyrins, which are currently being used as nanotweezers and nanograpples and these nanotools could be attached to the nanovehicles. The nanovehicles could potentially respond to chemical gradients and other factors such as light intensity and field gradients. Ultimately, the successful incorporation of sensor and reporter payloads might include informatics and cognition functions as well.



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